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Exploring the connectivity between the cerebellum and facial motor cortex

Dear Editor,

Transcranial magnetic stimulation (TMS) delivered over the cerebellum 5–7 ms prior to a stimulus applied over the contralateral M1 reduced the excitability of M1 corticospinal outputs [1]. This phenomenon, termed cerebellar brain inhibition (CBI) [1,2], has been interpreted as Purkinje cells stimulation leading to inhibition of deep cerebellar nuclei which itself has an excitatory disynaptic connection to primary motor cortex (M1) [3].

While CBI has been shown to exist for hand [4–6] and leg muscle representations [6,7], it remains unknown whether CBI is present in face M1 (fM1). In contrast to other body muscles, the muscles of the lower face receive a bilateral projection from fM1 [8], and therefore any cerebellar input might preferentially impact on brainstem structures that receive input from both hemispheres. Since previous studies utilizing CBI have provided insights into the pathophysiology of disorders involving the cerebello-thalamo-M1 tracts [3], we tested whether we could detect any evidence for CBI-fM1 using conventional methodology.

Fourteen right-handed healthy volunteers (9 females; 28.77 ± 1.11 years old) participated in this study (which was approved by the local ethics committee) after signing an informed consent. None of them had a history of neurological or psychiatric diseases or contraindications to TMS [9].

Electromyographic activity (EMG) was recorded from the right depressor anguli oris muscle (DAO) as reported by Pilurzi et al. [8]. Unrectified EMG signals were recorded (D360 amplifier, Digitimer Ltd, Welwyn Garden City, UK), amplified (x1000), filtered (bandpass 3–3000 Hz), sampled (5 kHz/channel; window frame length: 250 ms) using a power 1401 analog-to-digital converter (Cambridge Electronic Design, Cambridge, UK) and Signal 6.0 software on a computer and stored for off-line analysis.

TMS of fM1 was performed at the optimal spot using a figure-of-eight coil with external loop diameter of 7 cm connected to a Magstim 200 stimulator (Magstim Co., Whitland, Dyfed, UK) [8]. After determining the resting motor thresholds (RMT) [10] for the DAO, the test stimulus (TS) intensity was set to evoke an MEP in the relaxed muscle of at a least a 0.3 mV. Cerebellar stimuli (CS) were delivered with a 90 mm diameter double-cone coil connected to a Magstim 200 stimulator. We first tested whether CS of 70% of maximum stimulator output (MSO) activated any corticobulbar output by testing for the presence of an MEP or a possible silent period in the DAO during strong contraction. If there was no sign of brainstem activation, the CS were set to 50, 60 and 70% MSO as reported in previous studies [2,6]. The experiment was divided into two sessions that were performed two different days: central and lateral CBI. When assessing central CBI, the CS was delivered at the inion, while the CS for lateral CBI was delivered 3 cm lateral to the inion contralateral to the stimulated M1 [1,5].

In each session, three blocks with CS of 50, 60 and 70% MSO were randomized. In each block, 12 unconditioned and 12 conditioned MEPs were randomized. CS-TS pulses were paired at either 5 or 7 ms interstimulus intervals (ISIs). Amplitudes of conditioned and unconditioned responses were measured. CBI was expressed as the average MEP amplitude evoked by the cerebellar-conditioned stimulation of fM1 relative to the average MEP amplitude evoked by the unconditioned TMS pulses over fM1.

Statistical analysis was performed with SPSS 20 software (SPSS Inc, Chicago, IL, USA). Repeated measures (RM) analysis of variance (ANOVA) and planned post hoc *t*-test with Bonferroni correction for multiple comparison were used. Compound symmetry was evaluated with the Mauchly's test and the Greenhouse-Geisser correction was used when required. Significance was set for p value ≤ 0.05. Values are expressed as means ± standard error of the mean (SEM). To investigate the presence of CBI, a two-way RM-ANOVA, on MEP amplitude obtained during lateral stimulation, with ISI (TS, 5 and 7 ms), and INTENSITY (50%, 60%, 70% MSO) as within subject factors was used.

To examine the effect of stimulation site a two-way RM-ANOVA on the ratio using a 5 ms ISI, with SITE (central or lateral CS) and INTENSITY (50%, 60%, 70% MSO) as within subject factors was performed.

Mean RMT was 52.62 ± 2.93% MSO and the TS was 68.85 ± 4.33% MSO. None of the subjects had a silent period in the active DAO following CS at 70% MSO.

In the experiment using lateral stimulation a RM-ANOVA revealed a significant effect of ISI ($F_{2,24} = 3.403$, $p = 0.050$) but not INTENSITY ($F_{2,24} = 0.471$, $p = 0.630$) and no interaction among the factors ($F_{4,48} = 1.527$, $p = 0.233$). Specifically, post hoc analysis showed that the conditioned MEP was significantly smaller than TS only at 5 ms of ISI ($p = 0.014$), thus indicating a timing-specific effect of CBI on fM1 (Fig. 1A).

No significant differences in the amplitude of TS MEPs were detected with any CS intensity between the lateral (50%: 0.436 ± 0.07mV; 60%: 0.518 ± 0.10mV; 70%: 0.534 ± 0.07mV) and central (50%: 0.420 ± 0.05mV; 60%: 0.431 ± 0.06mV; 70%: 0.430 ± 0.05mV) stimulation sites.

When we compared the effect of stimulation site at the 5 ms ISI, a RM-ANOVA revealed a significant effect of CBI ratio for SITE ($F_{2,24} = 4.556$, $p = 0.054$) and a non-significant trend for INTENSITY ($F_{2,24} = 2.681$, $p = 0.089$). There was also a significant interaction ($F_{2,24} = 4.033$, $p = 0.038$), indicating that the effect of intensity

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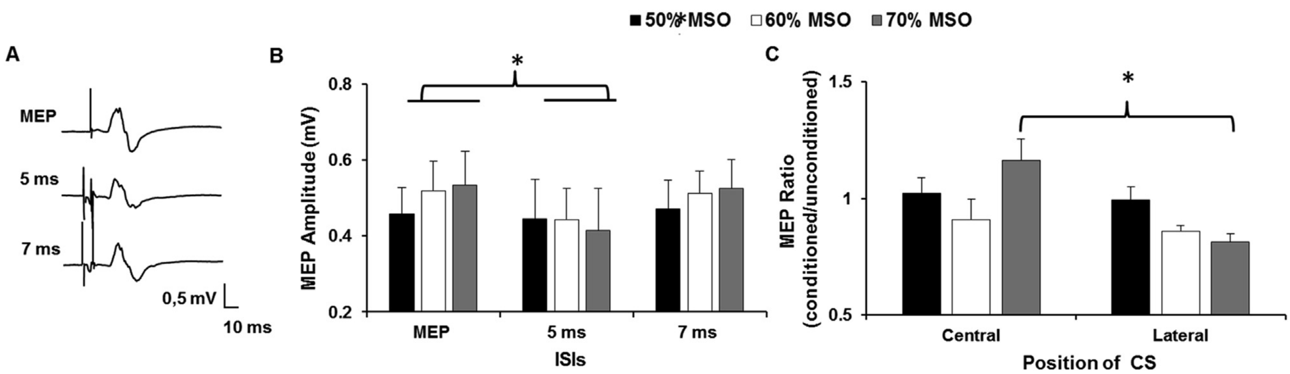

Fig. 1. Cerebellar Brain Inhibition in face primary motor cortex. A: Recordings of motor-evoked potentials (MEP) from the depressor anguli oris muscle (DAO) of a representative subject are reported for each condition (unconditioned MEP, induced by the test stimulus (TS), and conditioned MEPs at interstimulus intervals (ISIs) of 5 and 7 ms) with a conditioning stimulus of 70% maximal stimulator output (MSO) applied over the right lateral cerebellum. B: Effects of the TS alone and of the paired CS-TS at 5 and 7 ms ISIs and at different CS intensities on amplitude of the DAO MEP (mean ± standard error, SE). The conditioned MEP at 5 ms was significantly smaller than test MEP. C: Mean amplitude of conditioned DAO MEP (mean ± SE, expressed as ratio of the unconditioned MEP) at 5 ms ISI and different intensities of the CS over the central and lateral cerebellum. *$p < 0.05$.

differed between sites. Bonferroni analysis demonstrated that CBI was detectable only following lateral stimulation when using a ISI of 5 ms and a CS of 70% MSO ($p = 0.005$).

We report the first evidence of cerebellar–M1 connectivity for the representation of the DAO in healthy subjects. Similar to CBI for the hand and leg representations, the timing and location of cerebellar stimulation prior to probing cortical excitability was critical suggesting that the same pathways targeted with paired-pulse TMS for limb M1 extends to the cerebellar connection with fM1.

Limitations of this study are its exploratory nature and the small sample size. These preliminary data provide interesting insights into the understanding of the cerebellar control over facial muscles and may offer as a useful tool to detect somatotopic-specific effects of fM1-CBI when individuals learn new tasks involving facial muscles. Finally, it may be useful in order to better understand the involvement of cerebellum in the orofacial dystonia.

Conflicts of interest

The authors declare no conflicts of interest.

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